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FLY-ASH-INDUCED OXIDATIVE STRESS AND TOLERANCE IN *Prosopis juliflora* L. GROWN ON DIFFERENT AMENDED SUBSTRATES

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Abstract. Field experiments were conducted to study the impact of metal accumulation on malondialdehyde (MDA), cysteine and non-protein thiol (NPSH) contents in the plants of *Prosopis juliflora* grown on the fly ash (FA) amended with soil, blue green algae (BGA) biofertilizer, farm yard manure, press mud and *Rhizobium* inoculation. The analysis of data revealed that the level of MDA, cysteine and NPSH was higher in the roots of the plant than leaves, which was found positively correlated with metal accumulation. An increase of 361.14, 64.25 and 305.62% in MDA, cysteine and NPSH contents, respectively was observed after 45 days in the roots of the plants grown in 100% FA as compared to 100% garden soil (GS). The level of MDA, cysteine and NPSH was found less in the plants grown on various amendments of FA showing ameliorating effect on the toxicity induced due to the accumulation of metals. The decrease in MDA, cysteine and NPSH contents was higher in *Rhizobium*-inoculated plants as compared to uninoculated plants grown on 100% FA. The results showed a high tolerance potential of the plant, which is further increased by inoculating the plant with FA-tolerant *Rhizobium* showing feasibility of using *P. juliflora* in environmental monitoring of FA landfills.

Keywords: fly ash, *Prosopis juliflora*, *Rhizobium*, amendments

1. Introduction

Considerable attention has been paid to the management of coal combustion by-products such as fly ash (FA), which is disposed off in huge amounts because of the increase in coal utilization by thermal power plants. FA is accumulated in ash lagoons or landfills that has serious environmental impacts and require careful management methods in order to protect surrounding area. In this context, revegetation of FA lagoons could be a viable option to control erosion and leachate generation as well as for aesthetic rehabilitation purposes (Wong and Wong, 1990; Khan and Khan, 1996; Cheung *et al*., 2000; Vajpayee *et al*., 2000; Rai *et al*., 2004). However, the plants experience toxicity due to high pH, negligible N, P and high concentrations of many toxic metals (Cu, Zn, Mn, Pb, Hg, Cd, Ni, etc.) in the FA (Melhurn *et al*., 1989). The plants growing in these landfills have been found to accumulate high concentrations of toxic metals in their tissues (Mehra *et al*., 1998; Vajpayee *et al*., 2000; Rai *et al*., 2004). The bioaccumulation of toxic metals in various

cellular components is often accompanied by various physiological and biochemical changes due to high affinity of metals for sulphydryl groups. Metals have been demonstrated to induce free radical production and increased ion permeability due to both sulphydryl reaction and lipid peroxidation (De Vos *et al*., 1989; Halliwell and Gutteridge, 1993; Sinha *et al*., 1997). However, plants possess internal defense mechanism due to the presence of antioxidants and antioxidative enzymes to protect the damage caused by free radicals. Such plants under metal stress also synthesize metal-binding peptides (phytochelatins) showing high levels of non-protein thiols (NPSH) and cysteine (Grill *et al*., 1987; Zenk, 1996). However, such information for the plants growing in different FA amendments is meager (Kumar *et al*., 2002).

Plants of *Prosopis juliflora* L. (Leguminosae) have demonstrated potential to grow on FA ameliorated with a combination of various organic amendments, blue green algal biofertilizers and *Rhizobium* inoculation (Rai *et al*., 2004). Further, the plants growing in these amendments accumulated varied amounts of metals (Fe, Mn, Cu, Zn and Cr), however, *Rhizobium*-inoculated plants showed comparatively higher amounts of these metals with more translocation in to above ground parts. Considering such a variation in metal accumulation amongst various FA treatments, a study was planned to determine impact of metal accumulation on lipid peroxidation, cysteine and NPSH contents of *P. juliflora* and the results of the experiments is being reported in this paper.

2. Materials and Methods

2.1. Sample collection and preparation

Unweathered FA samples, collected from National Thermal Power Corporation, Unchahar, Raibareili, UP, India, were air dried before use in the study. Various amendments like farmyard manure (FYM) was procured from a commercial supplier, press mud (PM), from Biswan Sksaria Sugar Mill, Sitapur (UP) and BGA biofertilizer from UP Council of Science and Technology, Lucknow. The small saplings of exotic plant, *P. juliflora* L. growing under natural conditions was obtained from the Biomass Research Station, Banthara (National Botanical Research Institute, Lucknow) and acclimatized at least for 30 days under simulated field conditions in acid washed sand fortified with 10% Hoagland nutrient solution. The mature ripe pods were collected from the same site for the experiments involving inoculation of *Rhizobium*. The plant saplings were raised from mature seeds in 10% nutrient solution. Such exponentially growing plants were selected and used for experimental work.

2.2. Experimental design

The exponentially growing plants of *P. juliflora* were planted in 10 earthen pots in triplicate containing 5 kg of different amendments of FA with garden soil (GS)

(**A**); 10% FA + 90% GS (**B**), 25% FA + 75% GS (**C**), 50% FA + 50% GS (**D**), 75% FA + 25% GS (**E**), 100% FA (**F**)), BGA biofertilizer (**G**) (75% FA + 25% BGA) and other organic materials (75% FA + 25% FYM (**H**) and 75% FA + 25% PM (**I**)) for each exposure period and were grown in net-house under natural day light at 30 ± 4 °C with a photoregime of 14:10 h conditions. Plants growing in 100% GS served as control. For the inoculation of *Rhizobium* in 100% FA (**J**), strain PJ-1 was isolated from *P. juliflora* plants growing in FA-amended soils on Yeast Extract Mannitol Agar medium (Vincent, 1970). Before inoculation, the saplings of *P. juliflora* were raised in Jensen nitrogen-free medium (Jensen, 1942) and inoculated with 2 ml (12.8×10^8 cells ml^{-1}) of *Rhizobium* (PJ-1) culture. These plants were kept under normal growth conditions providing light intensity of 115 μmole m^{-2} s^{-1} for 14 h per day at 26 ± 2 °C for initiation and establishment of nodulation. Such nodulated plants were transferred to the pots containing 100% FA and kept under natural conditions. Uninoculated plants in 100% FA served as control for experiments involving *Rhizobium* inoculation. Plants were harvested after 30 and 45 days, repeatedly washed with double distilled water, separated into roots and leaves and analyzed for different parameters.

2.3. Cysteine estimation

Free cysteine content was measured in fresh plants by the method of Gaitonde (1967). Fresh plants (500 mg) were crushed in 3 ml 5% $HClO_4$ centrifuged at 10 000–15 000 rpm for 10 min. To a 0.5 ml aliquot, 0.5 ml of acetic acid and 0.5 ml of acid ninhydrin reagent were added and heated on the water bath covered with aluminum foil for 10 min. Absorbance was read at 560 nm.

2.4. Lipid peroxidation estimation

The level of lipid peroxidation was measured in terms of malondialdehyde (MDA) content as estimated by thiobarbituric acid reaction (Heath and Packer, 1968). Plant samples (500 mg) were homogenized in 3 ml, 0.2% TCA. The homogenate was centrifuged at 10 000–15 000 rpm for 10 min. To a 1 ml of aliquot, 4 ml 20% TCA containing 0.5% TBA was added. The mixture was heated at 95 °C for 30 min and cooled in an ice bath for 2–3 h. After centrifuging again at 10 000 rpm for 10 min, the absorbance of the supernatant was read at 532 nm. The concentration of MDA was calculated using the extinction coefficient of 155 $mM^{-1}cm^{-1}$ and by correcting for the a specific absorbance at 600 nm (532–600 nm).

2.5. Non-protein thiol estimation

NPSH content was measured following the method of Ellman (1959). Plant samples (500 mg) were homogenized with 6.67% of 5-sulfosalicylic acid (3 ml) and kept in ice for 10 min. The mixture was centrifuged at 10 000–12 000 rpm at 4 °C for

10 min. To a 1 ml aliquot of the supernatant, 5 ml of Ellman reagent was added and absorbance was measured at 412 nm.

2.6. Statistical analysis

The experiments were performed in a completely randomized block design involving 10 amendments of FA with GS, organic materials, BGA and *Rhizobium* at two durations. To confirm the variability of data and validity of results, all the data were subjected to analysis of variance (ANOVA). The correlation coefficient (r) significant at 5% level ($p < 0.05$) was determined between various physiological parameters (MDA, cysteine and NPSH) and metal accumulation in both parts of the plant growing in different FA amendments after 45 days of exposure period. To determine the significant difference between treatments, Duncan's multiple range test (DMRT) was performed (Gomez and Gomez, 1984).

3. Results and Discussion

3.1. MDA content

The accumulation of heavy metals in the plants of *P. juliflora* grown in different FA amendments has recently been reported by our group (Rai *et al*., 2004). The plants grown on different amendment of FA-accumulated metals in order Fe > Cu > Mn > Zn > Cr, however, their accumulation was more in roots than shoots in all the amendments. The *Rhizobium*-inoculated plants of *P. juliflora* also showed significantly high amounts of these metals in roots than shoots except accumulation of Fe which was high in shoot (Rai *et al*., 2004). Increased accumulation of metals induced lipid peroxidation in both leaves and roots of the plants as measured by MDA content (Table I). Maximum content of MDA (74.06 μ mole g^{-1} fw) was observed in the roots of the plant grown in 100% FA after 45 days followed by 75% FA + 25% GS, 50% FA + 50% GS, 25% FA + 75% GS and 10% FA + 90% GS. However, 75% FA either with BGA, FYM and PM showed lower contents of MDA as compared to 75% FA + 25% GS, showing ameliorating effect of these amendments on lipid peroxidation induced due to metal accumulation. Further, inoculation of plant with FA-tolerant *Rhizobium* (PJ-1) strain resulted into protection of lipid peroxidation (over 50%) than the uninoculated plants grown in 100% FA conditions (DMRT, $p < 0.05$). In plants, metals have high potential to promote oxygen-free radicals production and thus lead to peroxidation of membrane lipids (De Vos *et al*., 1989; Sinha *et al*., 1997; Somshekaraiah *et al*., 1992). Kumar *et al*., (2002) also reported an increase in MDA content in *Cassia siama* grown in different FA amendments due to accumulation of metals. The results of the present study conform to the finding of these authors. However, an increase in MDA content in some amendments was insignificant (DMRT, $p < 0.05$) which might be due to

TABLE I
Effect of various fly ash amendments on MDA content in different plant parts of *P. juliflora* at different treatment durations

	MDA (μ mole g^{-1} fw)			
	Leaves		Roots	
Treatments	30 days	45 days	30 days	45 days
A	6.13 ± 0.39	10.55 ± 0.83	9.30 ± 0.62	16.06 ± 0.99^{c}
B	8.69 ± 0.66^{g}	15.28 ± 0.99	12.66 ± 0.86^{c}	20.50 ± 1.15^{a}
C	10.04 ± 0.75^{fg}	18.08 ± 1.28	18.41 ± 1.42^{a}	32.12 ± 1.67
D	12.28 ± 0.89^{de}	27.52 ± 1.96^{b}	38.5 ± 2.25	53.56 ± 3.81
E	15.20 ± 0.97^{ab}	37.26 ± 2.36^{a}	48.74 ± 3.49	66.68 ± 3.95
F	20.46 ± 1.44	56.89 ± 3.22	62.28 ± 3.87	74.06 ± 4.53
G	11.35 ± 0.85^{ef}	28.98 ± 1.98^{b}	12.71 ± 0.94^{c}	18.10 ± 0.94^{bc}
H	12.58 ± 0.94^{cd}	33.36 ± 2.46	18.01 ± 1.10^{ab}	20.08 ± 1.34^{ab}
I	14.19 ± 0.89^{abc}	37.4 ± 2.04^{a}	20.96 ± 1.49	24.25 ± 1.85
J	15.87 ± 1.08^{a}	45.04 ± 3.17	17.68 ± 1.39^{ab}	37.78 ± 2.96

Mean $\pm$ SD ($n = 3$), ANOVA $p < 0.05$. Identical superscripts on values denote non-significant difference ($p < 0.05$) between means of different treatment according to Duncan's multiple range test.

enhanced level of antioxidants, however, the responses of antioxidants varied with plant species and metal involved (Mazhoude *et al*., 1997).

3.2. Cysteine and non-protein thiol contents

The analysis of the data presented in Table II showed an increase in cysteine content in both roots and leaves of the plant grown on various FA amendments and increased with increase in FA/amendment ratio. It was found maximum (2139.26 μmole g^{-1} fw) in root tissues of the plant grown on 100% FA after 45 days (DMRT, $p < 0.05$). At 45 days, its minimum concentration (690.03 μmole g^{-1} fw) was found in the roots of the plants grown in 25% BGA + 75% FA. Cysteine content in root and leaf tissues of the plant grown in 75% FA amended with organic materials (FYM, PM) showed that the level of induction of cysteine in the plant decreased as compared to 75% FA + 25% GS. However, by inoculating the plant with *Rhizobium* (PJ-1) in 100% FA, the cysteine accumulation inside the plant has decreased showing toxicity amelioration in the plant to grow under 100% FA condition (DMRT, $p < 0.05$). Treatment duration increased the content of cysteine in all the amendments.

Cellular concentrations of various toxic metals (Rai *et al*., 2004) increased NPSH, an index of phytochelatins, in the plant grown in different FA amendments (Table III). Maximum accumulation of NPSH was recorded in the plant grown

TABLE II
Effect of various fly ash amendments on cysteine content in different plant parts of *P. juliflora* at different treatment durations

	Cysteine (μ mole g^{-1} fw)			
	Leaves		Roots	
Treatments	30 days	45 days	30 days	45 days
A	198.03 ± 9.93^{a}	279.59 ± 17.53^{c}	389.70 ± 18.90^{c}	1302.21 ± 68.17
B	213.09 ± 14.91	359.73 ± 16.83^{b}	422.82 ± 32.56^{c}	1371.32 ± 73.86
C	237.84 ± 16.57	439.66 ± 25.76	664.66 ± 37.1^{ab}	1554.69 ± 40.62
D	324.03 ± 13.65	565.56 ± 39.82	711.37 ± 42.10^{a}	1648.76 ± 80.89^{a}
E	418.59 ± 23.11	686.76 ± 32.10^{a}	933.93 ± 54.66	1760.99 ± 84.28
F	523.97 ± 26.95	727.22 ± 43.32	1166.63 ± 67.59	2139.26 ± 104.8
G	141.47 ± 8.46	290.72 ± 18.16^{c}	151.86 ± 11.21	690.03 ± 37.30
H	159.83 ± 13.19	344.16 ± 15.36^{b}	305.74 ± 17.09	945.09 ± 48.25
I	208.90 ± 14.6^{a}	400.52 ± 28.41	512.15 ± 28.66	1179.07 ± 69.81
J	362.11 ± 27.91	673.67 ± 40.49^{a}	687.06 ± 42.5^{ab}	1680.39 ± 89.21^{a}

Mean $\pm$ SD ($n = 3$), ANOVA $p < 0.05$. Identical superscripts on values denote non-significant difference ($p < 0.05$) between means of different treatment according to Duncan's multiple range test.

TABLE III
Effect of various fly ash amendments on NPSH content in different plant parts of *P. juliflora* at different treatment durations

	NPSH (μ mole g^{-1} fw)			
	Leaves		Roots	
Treatments	30 days	45 days	30 days	45 days
A	63.99 ± 6.21^{d}	161.14 ± 8.92	146.10 ± 9.29^{c}	346.86 ± 24.14
B	91.78 ± 8.37^{abc}	234.20 ± 17.04^{b}	187.86 ± 12.22	403.48 ± 28.21
C	108.19 ± 9.17^{a}	287.71 ± 18.58^{a}	239.01 ± 15.04	501.40 ± 31.87^{b}
D	236.63 ± 14.76	402.96 ± 23.84	361.16 ± 21.66^{b}	636.34 ± 38.50
E	266.14 ± 17.39	532.32 ± 30.93	414.47 ± 27.16^{a}	742.22 ± 47.69^{a}
F	378.61 ± 21.13	640.34 ± 32.64	618.60 ± 36.09	1406.94 ± 82.96
G	45.71 ± 3.83^{e}	187.96 ± 20.31	131.15 ± 7.26^{c}	438.99 ± 26.02
H	60.92 ± 4.64^{de}	224.66 ± 13.17^{b}	283.90 ± 19.49	521.14 ± 32.43^{b}
I	76.94 ± 5.08^{cd}	294.06 ± 15.32^{a}	356.73 ± 22.36^{b}	762.78 ± 43.54^{a}
J	103.42 ± 8.1^{ab}	328.26 ± 19.38	424.61 ± 27.38^{a}	860.68 ± 49.59

Mean $\pm$ SD ($n = 3$), ANOVA $p < 0.05$. Identical superscripts on values denote nonsignificant difference ($p < 0.05$) between means of different treatment according to Duncan's multiple range test.

in 100% FA (DMRT, $p < 0.05$), which increased with the increase in treatment duration. The concentration of NPSH was approximately two fold in root tissues than leaves at both the treatment durations and all FA amendments. Similar to cysteine content, the NPSH content also decreased in the plants grown on 75% FA amended with 25% BGA or FYM as compared to 75% FA + 25% GS, however, nonsignificant change was found in 75% FA + 25% PM. In contrast, the plants grown on 100% FA inoculated with *Rhizobium* have shown decrease in accumulation of NPSH (DMRT, $p < 0.05$) in both the tissues, suggesting protective role of *Rhizobium* against metal toxicity. The NPSH content was minimum in the plant grown in GS (control).

Metals have relatively high affinity for cellular sulfhydryl groups. Among thiol compounds, glutathione (GSH) is the most important free thiol, which plays a prominent role in defense against free radicals. Cysteine constitutes one of the amino acids of GSH. These antioxidants play important role in inducing resistance to metals by protecting labile macromolecules against attack by free radicals which are formed during various metabolic steps leading to oxidative stress (Galli *et al*., 1996; Rauser, 1987; Halliwell and Gutteridge, 1993). The results obtained during present study showed an increase in NPSH and cysteine contents in the root and leaves of the plants grown at different levels in various FA amendments. The increase content of these metabolites corresponds to the tolerance exhibited by metal-treated plants (Rai *et al*., 1995; Sinha *et al*., 1997). The results of the present study agree with the findings of Nussbaum *et al*. (1988), showing an increase in the level of cysteine content in the roots of *Zea mays* at a lower level of Cd and a decrease at higher concentrations, which was due to depletion of metal in the solution. Since NPSH content is considered to be indicator of phytochelatin (PC) synthesis in plants, it is quite possible that plant of *P. juliflora* grown in various FA amendments might have synthesized PCs to account for detoxification of metals as reported in case of *C. siamia* (Kumar *et al*., 2002).

3.3. Correlation coefficient

MDA, cysteine and NPSH contents increased in both roots and leaves of the plants grown in different FA amendments, therefore, it was considered desirable to study correlation between metal accumulation and induction of these variables. The data presented in Tables IV and V show the correlation coefficient values of metal accumulation with MDA, cysteine and NPSH contents in both tissues, which was found positively correlated with metal accumulation in the plant. However, a comparative analysis of the data amongst various treatments showed different trend. Iron accumulation in roots of 100% FA and 100% FA *Rhizobium*-inoculated plant was found positively correlated with MDA, cysteine and NPSH contents, while in case of BGA, FYM and PM amendments, it was negatively correlated. In contrast, shoot Fe content is positively correlated with these parameters in case of 100% FA-grown plants. These parameters were also found positively correlated with Mn

TABLE IV

Correlation coefficient of various physiological parameters studied in different plant parts with metal accumulation in root of *P. juliflora* grown in different fly ash amendments after 45 days

	Cysteine		MDA		NPSH	
Metals	Leaf	Root	Leaf	Root	Leaf	Root
Fe	0.837[a]	0.799[a]	0.787[a]	0.788[a]	0.830[a]	0.729
	0.048	*−0.938*[a]	*0.612*[a]	*0.204*	*0.155*	*0.175*
	0.644[a]	**0.921**[a]	**0.743**[a]	**0.951**[a]	**0.960**[a]	**0.905**[a]
Mn	0.952[a]	0.956[a]	0.978[a]	0.959[a]	0.981[a]	0.926[a]
	0.245	*−0.866*[a]	*0.749*[a]	*0.395*	*0.349*	*0.367*
	0.836[a]	**0.498**	**0.753**[a]	**0.420**	**0.393**	**0.531**
Cu	0.991[a]	0.891[a]	0.905[a]	0.983[a]	0.972[a]	0.798[a]
	0.467	*−0.771*[a]	*0.904*[a]	*0.560*[a]	*0.522*	*0.512*
	0.954[a]	**0.716**[a]	**0.904**[a]	**0.652**[a]	**0.630**	**0.742**[a]
Zn	0.996[a]	0.948[a]	0.941[a]	0.988[a]	0.399	0.867[a]
	0.426	*−0.728*[a]	*0.849*[a]	*0.476*	*0.444*	*0.412*
	0.814[a]	**0.463**	**0.725**[a]	**0.383**	**0.356**	**0.496**
Cr	−0.811[a]	−0.624	−0.595	−0.825[a]	−0.727	−0.458
	0.801[a]	*−0.223*	*0.887*[a]	*0.776*[a]	*0.769*[a]	*0.724*[a]
	−0.915[a]	**−0.998**[a]	**−0.962**[a]	**−0.990**[a]	**−0.985**[a]	**−0.999**[a]

Comparisons were made amongst treatment 100% GS and 100% FA (normal; $n = 5$); 100% GS, BGA, FYM and PM (italics; $n = 11$); 100% GS, 100% FA and 100% FA + *Rhizobium*-inoculated plants (bold; $n = 8$).

[a]Significant at $p < 0.05$.

contents in both root and leaf tissues of the plant grown in 100% FA, and cysteine and MDA contents in leaves of 100% FA *Rhizobium*-inoculated plants. Similarly, these variables were found positively correlated with the Cu content in both root and leaf tissues except in case of BGA, FYM and PM amendments, which is negatively correlated with the metal accumulation. However, leaf Cu concentration in case of MDA and cysteine in roots and NPSH in both tissues was negatively correlated in *Rhizobium*-inoculated plants, which may be due to toxicity at higher metal levels. The accumulation of Zn by both the tissues were also found positively correlated with MDA, cysteine and NPSH induction in case of 100% FA and *Rhizobium* inoculated 100% FA grown plants, while it was negatively correlated in the plants grown in BGA, FYM and PM amendments. A reverse trend in correlation coefficient was obtained in case of Cr, which was found negatively correlated with these parameters in both the tissues. However, root Cr content has a positive correlation with MDA and NPSH in both the tissues and with leaf cysteine in the plants grown in BGA, FYM and PM amendments.

TABLE V

Correlation coefficient of various physiological parameters studied in different plant parts with metal accumulation in leaves of *P. juliflora* grown in different fly ash amendments after 45 days

	Cysteine		MDA		NPSH	
Metals	Leaf	Root	Leaf	Root	Leaf	Root
Fe	0.839[a]	0.879[a]	0.854[a]	0.800[a]	0.857[a]	0.839[a]
	−0.462	*−0.960*[a]	*0.162*	*−0.335*	*−0.381*	*−0.370*
	0.644[a]	**0.228**	**0.531**	**0.141**	**0.112**	**0.264**
Mn	0.989[a]	0.915[a]	0.905[a]	0.976[a]	0.966[a]	0.819[a]
	0.235	*0.904*[a]	*−0.350*	*0.176*	*0.213*	*0.242*
	0.893[a]	**0.592**	**0.822**[a]	**0.519**	**0.494**	**0.623**
Cu	0.987[a]	0.897[a]	0.920[a]	0.989[a]	0.975[a]	0.822[a]
	−0.287	*−0.986*[a]	*0.336*	*−0.143*	*−0.192*	*−0.177*
	0.871[a]	**0.554**	**0.794**[a]	**0.479**	**0.453**	**0.586**
Zn	0.660	0.761[a]	0.775[a]	0.608	0.728[a]	0.786[a]
	−0.871[a]	*0.304*	*−0.987*[a]	*−0.926*[a]	*−0.908*[a]	*−0.903*[a]
	0.919[a]	**0.640**[a]	**0.855**[a]	**0.570**	**0.546**	**0.669**[a]
Cr	−0.026	0.067	−0.079	−0.079	−0.057	−0.021
	0.201	*−0.484*	*0.506*	*0.157*	*0.144*	*0.079*
	0.467	**0.016**	**0.340**	**−0.072**	**−0.101**	**0.054**

Comparisons were made amongst treatment 100% GS and 100% FA (normal; $n = 5$); 100% GS, BGA, FYM and PM (italics; $n = 11$); 100% GS, 100% FA and 100% FA + *Rhizobium*-inoculated plants (bold; $n = 8$).

[a]Significant at $p < 0.05$.

4. Conclusion

Plants of *P. juliflora* growing in different amendments of FA exhibited high levels of MDA, cysteine and NPSH, which was more in roots. An increase of 361.14, 64.25 and 305.62% in MDA, cysteine and NPSH contents, respectively was observed after 45 days in the roots of the plants grown in 100% FA as compared to 100% GS. Overall analysis of the data showed the decrease in MDA, cysteine and NPSH contents in the plants grown on FA amended with the BGA, FYM and PM as compared to the plants grown on FA amended soil showing ameliorating effects of these amendments. Metal accumulation in the plant was found positively correlated with these parameters. Although, various amendments of FA were found suitable for amelioration of FA toxicity, inoculation of FA-tolerant *Rhizobium* to the plants may prove to be a good tool for environmental monitoring and management of FA dykes through initiating revegetation programmes. Further, the results obtained during the present study revealed that *P. juliflora* is a suitable material for plantation

of FA landfills as it has adequate tolerance and potential detoxification mechanism for metals.

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